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Recent advances in marine microbiology - a selection of critical evaluations of research papers

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Recent Advances in Marine Microbiology - a Selection of Critical Evaluations of Research Papers

Introductory remarks by Dr Colin Munn

This collection of articles features examples of coursework submitted by three students taking the module BIOL3309 'Marine Microbiology – Ecology & Applications' as part of the BSc Hons Marine Biology degree at Plymouth. In this module, students discuss recent research papers in regular seminars, and keep a portfolio of critical evaluations of papers that they have studied. This subject is arguably one of the fastest moving fields in marine science and recent discoveries, made possible by the application of new techniques (especially metagenomics and rapid DNA sequencing) have revolutionized our understanding of ocean processes and the interactions of microbes with other marine life. Thus, students taking this module are encouraged to monitor the latest issues of journals and to select papers of their own choice, so that they gain an appreciation of the most recent advances. Students are provided with detailed advice on approaches to studying scientific literature and are given a template to structure their evaluations. There are no hard and fast rules on the style of evaluations or the number of papers or the level of detail needed, but students obtaining top grades normally submit detailed analyses of at least 2 or 3 papers in each of the 5 or 6 major themes of the course. The value of this assignment is appreciated by most students, although it is considered by some to be onerous, as evidenced in these representative comments from other students taking the module.

- “Engages you in the scientific literature”.
- “Makes you assess good and bad science, helping you improve your own studies”.
- “I considered the portfolio to be a very long and time consuming piece of coursework ... however, I thought the portfolio was very useful in terms of keeping up to date with recent scientific advances and I found it particularly useful with the exams”.
- “A lot of coursework for only 30% of the module ... [but it] increases one’s research interests”.
- “I think it is very interesting coursework that pushes students to search and learn more on an area of interest. I took great pleasure to compile and criticize articles found. The fact that we are not experienced scientists gives another look to that process of critique. We do not know if the articles are significant or not, but at least we try to give an opinion.”

The evaluations presented in this article have been selected because they are exceptionally insightful and detailed and to illustrate some exciting recent developments in marine microbiology. They provide an interesting perspective on different ways in which undergraduate students approached this task and how they perceive science at the cutting edge.

New Insights into Utilization of Nutrients by Marine Bacteria

Heather Guy

A review of the article:

Stocker, R., Seymour, J.R., Samadani, A., Hunt, D.E., Polz, M.F. (2008) Rapid chemotactic response enables marine bacteria to exploit ephemeral microscale nutrient patches. Proceedings of the National Academy of Sciences of the United States of America **105**: 4209-4214.

Summary: Marine bacteria inhabit a complex environment where nutrients are typically concentrated in small, often ephemeral, patches that can contain labile organic compounds at concentrations two to three orders of magnitude above ambient seawater. Although these individual patches of dissolved organic matter [DOM] are small, they are abundant, and therefore bacteria may gain significant growth advantages if they are able to exploit them. In addition, the efficiency of marine bacteria to exploit such patches would exert a fundamental control on biochemical transformation rates in the ocean. However, prior to the publication of this study, it had never been quantitatively evaluated if the chemotactic response of marine bacteria is rapid enough to facilitate significant gain from nutrient patches in the face of their rapid physical dissipation. The key question addressed by this study, therefore, was: to what extent are marine bacteria capable of exploiting ephemeral nutrient patches generated at experimentally realistic spatiotemporal scales?

Utilizing microfluidic devices, two types of patches expected to occur widely in the ocean were generated: a purely diffuse pulse (indicative of lysed algae) and a plume governed by diffusion and advection (indicative of sinking organic particles). Through the simultaneous measurement of the spatiotemporal distribution of nutrients and cells, the authors quantified the nutrient exposure experienced by the chemotactic marine bacterium *Pseudoalteromonas haloplanktis*. They report that the rapid chemotactic response of this marine bacterium substantially enhances its ability to exploit nutrient patches before they dissipate. The accumulation of *P. haloplanktis* in response to a nutrient pulse led to a formation of bacterial hotspots within tens of seconds, resulting in a ten-fold higher nutrient exposure for the fastest 20% of the population compared with nonmotile cells. Furthermore, the chemotactic response of *P. haloplanktis* was >10 times faster than the classic chemotaxis model, *Escherichia coli*, leading to twice the nutrient exposure. The authors successfully demonstrate that such a rapid response enables *P. haloplanktis* to colonize nutrient plumes at realistic particle sinking speeds, with up to a 4-fold nutrient exposure compared with nonmotile cells. The authors propose that the chemotactic swimming strategies of marine bacteria in patchy nutrient seascapes exert strong influence on carbon turnover rates by triggering the formation of microscale hotspots of bacterial productivity.

Style: The purpose of the article is to communicate concisely to the scientific community that marine bacteria have a chemotactic capability which enables their utilization of small ephemeral nutrient patches. The study employs realistic conditions to accurately simulate natural responses in the field. The article combines physical theory (fluid model) with biology (chemotaxis of a marine microorganism) for an informed scientific study. I believe such integrative approaches are typically not utilized enough within the scientific community, with researchers typically constrained within their particular field of interest. The style of writing is targeted at readers with a background in biology, with physical theory explained clearly and in a simple manner (with the exception of some numerical equations which require careful consideration). The layout of the paper is typical of the journal in which it is published, including summary, introduction, results, discussion, and methodology sections. Figures are colourful and eye-catching to readers, but also communicate clear scientific findings.

Objectivity/credibility/authority: This study was published within a high-status peer-reviewed journal. The first author of the paper, Roman Stocker, is an assistant professor and principle investigator within the 'Environmental Microfluids Group' in the Department of Civil and Environmental Engineering, Massachusetts Institute of Technology. He has a vast array of publications and a strong background in engineering and mathematics (see web.mit.edu/romanstocker/ for more details). The Massachusetts Institute of Technology is highly prestigious, boasting a top-class undergraduate engineering programme that was ranked the best in the U.S. in 2007 (U.S. News & World Report's annual rankings guide, see web.mit.edu/ for more details). All aforementioned points imply high academic authority and credibility. Work was financially supported by a number of grants, including one from the National Science Foundation [NSF], and one from the Department of Energy 'Genomes to Life' Programme. The goal of the 'Genomes to Life' Programme is to "gain insights about fundamental biological processes and, ultimately, a predictive understanding of how living systems operate" (see genomicsgtl.energy.gov/ for more details). The gain of financial support from non-commercial sources suggests no bias in reported results, with no particular outcome preferred over another. In addition, in general, an objective tone was assumed throughout the report.

Methodology and Results: Briefly, methodology included the creation of microfluidic channels that were 45 mm long, 3 mm wide, and 50 μm deep. Two in-line inlets separately introduced bacteria and nutrients. For 'plume' experiments a cylinder was added in front of the nutrient outlet to disperse the nutrients (as opposed to introducing them in one thin, straight line). Cultures of *P. haloplanktis* within their exponential growth phase were obtained, diluted 1:20 and starved for 72 hours prior to the experiment. The chemoattractant (i.e. the nutrients) consisted of culture filtrates of the marine phytoplankton *Dunaliella tertiolecta*. The nutrient plumes were visualized utilizing fluorescein, and experiments were viewed and recorded using an inverted microscope. Mathematical modelling was also performed to predict chemotactic exploitation of three dimensional patches in the field. Methodology is clearly explained, although a disadvantage of the procedures being featured at the end of the paper is that they have to partially be explained *again* in the results section. Potential limitations of the study include the fact that the 'plume' and 'sinking particle' were simulated on a horizontal plane, whereas in the ocean, DOC would more likely be descending vertically through the water column, or would perhaps be buffered in more than one direction if it is near the surface. Therefore,

although the experiment provided a more accurate representation of particle movements than previous studies, the results are unlikely wholly representative of true oceanic conditions. In addition, *P. haloplanktis* was completely starved for 72 hours prior to the experiments, and it is also unclear if this is representative of natural conditions, or if in fact this specific bacterial species normally utilizes the consistent low background levels of nutrients present within the ocean. To confirm how either of these criticisms may have affected the results, however, further research is required. The findings may also only be representative of chemotactic responses when these specific bacteria and algae are examined. Thus, further work also needs to be completed to confirm the applicability of these results to other microbial groups.

Results are described concisely, and are well illustrated by appropriate figures. However, numerical equations are included which require much consideration by the reader to fully understand the dynamics of the findings. The results section is large, taking up the majority of the space in the article; an overview of the key findings is given below:

Nutrient pulse (one dimensional): In brief, *P. haloplanktis* cells aggregated within the centre of the nutrient band within tens of seconds, where they then remained (as a hotspot) for up to 15 minutes. The experiment was repeated with *E. coli*, which displayed a markedly slower chemotactic response to two of its most potent chemoattractants at near-optimal concentrations. Comparisons revealed that *P. haloplanktis* was more than 10 times faster at chemotaxing into the nutrient patch than *E. coli*, which is partly reflective of a difference in mean swimming speed. Motility significantly enhanced the nutrient exposure experienced by the cells. It is suggested the one dimensional microscale experiments provided a conservative estimate of chemotactic advantage in the three dimensional ocean.

Nutrient plume (two dimensional): At a slow sinking rate ($66 \mu\text{m s}^{-1}$) bacteria accumulated immediately behind the 'particle' (cylinder) and increased down the stream, creating a hotspot of bacteria inside the plume where bacterial concentration was 4 times of that outside of the plume. As sinking rate increased, however, the chemotactic responses did not lead to the creation of hotspots, and although bacteria moved into the plume, it was to a much lesser extent. They conclude, based on mathematical modelling, that chemotaxis would also be of advantage with regard to nutrient exposure within three dimensional plumes in an ocean setting.

Arguments and Conclusions: The authors conclude that the findings may help explain bacterial patchiness in the ocean. They conclude that the short response time enabled *P. haloplanktis* to reach cell concentrations three times above background values well before patches were eroded by diffusion (conferring a 3-fold advantage over nonmotile cells). These findings may be an overestimation, as natural nutrient patches may not act as such strong chemoattractants. However, these findings are in-line with previous estimates (see Blackburn et al. 1998), suggesting they are accurate. Plume colonization depended on sinking speed; however, chemotactic response to the plume resulted in a significant nutrient exposure advantage over nonmotile cells for all sinking speeds. Modelling confirmed an increase in chemotactic advantage with speed, yet to a smaller degree than observed in the experiments, suggesting that *P. haloplanktis* relies on behavioural strategies beyond speed to exploit patches. For example, they may employ a "run

and reverse” swimming strategy (see Johansen et al. 2002). Further work is required to determine exact behavioural strategies, although the authors do not make any explicit suggestions for future studies themselves.

Significance: Prior to the current study, colonization of nutrient plumes by marine bacteria had been postulated, but never observed. The authors clearly recognize the potential significance of their findings and state that “the results presented here strongly suggest that marine bacteria are capable of responding to ephemeral nutrient patches of organic matter before these patches dissipate, indicating that bacterial chemotactic behaviour in the ocean may impact planktonic trophodynamics and biogeochemical transformation rates”. Indeed, widespread plume colonization could increase carbon turnover rates (see Azam & Long 2001), having potentially huge implications for carbon cycling within the ocean. Despite publication only last year (March 2008), the article has already been cited five times to date (23/03/09). Three of the articles report research regarding bacterial motility and one paper is a ‘research highlight’ type piece, which evaluates microscale technologies. The final citation was within an article which discussed patch formation and exploitation by marine microbes, and attempted to quantify swimming behaviours of *P. haloplanktis* (Seymour et al. 2009). This is likely a direct follow-up to the paper discussed here, as a need for quantification of swimming behaviour, although not explicitly stated, was hinted at, and the last author of the paper by Seymour et al. (2009) was Roman Stocker himself.

Connections: It has recently been suggested that ocean bacteria follow at least two different adaptive strategies: the “opportunitroph” actively exploiting patchy sources, and the passive oligotroph, efficiently using low nutrient concentrations (Polz et al. 2006). The results of the paper by Stocker et al. (2008) provide evidence that *P. haloplanktis* is an “opportunitroph”, which contrasts with the ubiquitous marine bacteria *Pelagibacter ubique*, which does not respond to nutrient addition, suggesting it is a passive oligotroph. The genome of *P. ubique* is much reduced, suggesting it makes use of the ambient DOC field, whereas heterotrophic bacterioplankton with larger genomes (likely such as *P. haloplanktis*) are capable of exploiting nutrient pulses, as they have the machinery required to implement a variety of metabolic strategies (see the paper by Giovannoni et al. 2005 for more details about *P. ubique*). From reviewing these two articles, it was made clear to me that microorganisms have likely employed many differential adaptive strategies for survival in the marine environment. To further my understanding of how marine microbes exploit their habitat and survive, I now need to research the varying strategies employed by different microorganisms in the marine realm. I can then compare and contrast my findings for a comparative overview of this complex topic area.

Overall judgement and other comments: This article concisely reports a solid piece of scientific research that was carried out by credible and authoritative academics. It successfully integrates the disciplines of mathematics, physics, and biology for the brilliant outcome of a truly intelligent model of bacterial chemotaxis in oceanic conditions.

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First Experimental Use of Pyrosequencing Reveals Effect of Environmental Stress on Coral Viruses

Grace Phillips

A review of the article:

Thurber, R. L. V., Barott, K. L., Hall, D., Liu, H., Rodriguez-Mueller, B., Desnues, C., Edwards, R. A., Haynes, M., Angly, F. E., Wegley, L. and Rohwer, F. L. Metagenomic analysis indicates that stressors induce production of herpes-like viruses in the coral *Porites compressa*. Proceedings of the National Academy of Sciences of the United States of America 105: 18413-18418.

Summary: An increase in viral particles within dinoflagellates has been hypothesised to be responsible for symbiont loss during bleaching. To date, no coral viral diseases have been positively identified, but pathologies such as white band disease and yellow blotch are hypothesised to be the results of viral infections. Several families of virus-like particles (VLPs) have been visually identified on four scleractinian coral species. Environmental conditions were manipulated to determine whether environmental stressors induce viral diseases. The results suggest that a distantly related herpes-like virus or a group of viruses is commonly associated with corals and Cnidarians in general and are produced as a result of environmental stress.

Methods:

- Specimens of coral *Porites compressa* were collected in the Hawaii Marine Laboratory Refuge.
- Specimens were exposed to thermal, pH, nutrient, and dissolved organic carbon stress for 64 hours. Pyrosequencing was conducted.
- Sequences were compared using BLASTn.
- Each sequence library was analysed for the total number of sequence similarities to each of the eukaryotic viral genomes using tBLASTx.
- To determine temporal changes in viral abundance during manipulation experiments, PSI-BLAST was used.
- A 2nd experiment was conducted 1 year later to test the possibility that the viruses discovered in the corals were unique to the original experiment and/or the result of unnatural stress conditions.

Results:

- 19 (out of ~100) known viral families were found in the coral-associated viromes.
- A large proportion of the best sequence similarities in the stressed samples were related to the *Herpesviridae* family, with the nutrient, pH, and temperature- stressed samples containing 64%, 53%, and 70% herpes-like sequences respectively.

- The host range of coral-associated viruses was shown to be wide, suggesting that they have many possible hosts including metazoans, protists, fungi and plants.
- The % of viral similarities to *Herpesviridae* rose from ~19% in the control to 30%, 32% and 32% for the temperature, nutrient, and pH stressed samples respectively. The increase in herpes-like virus was confirmed by the 2nd experiment.

Style: The paper was generally difficult to read (see 'Methods and Results' below). Although the long 'Results and discussion' section was split up with subheadings, I still feel that the section was too long. They do however finish up with quite a long conclusion, which I feel is necessary in order to provide a useful summation of the results/discussion. The language used was not complicated, and the tables and graphs displayed in the paper helped to convey the results. This paper has received no citations as yet, however it has only been published for three months, and certainly, due to its significance, it will most likely start receiving citations in the coming months.

Methods and results: The methods section was drastically lacking in information. It (briefly) gave only the molecular methods used and not the general information about how the study was conducted. The general and more detailed methodological information was included in with the 'Results and discussion' section, which I feel would have been more appropriately titled 'Methods, results and discussion'. Due to the inclusion of all three sections into one, the paper became difficult to read. The reader did not know what was happening in advance, because all the methods were not stated beforehand, thus when the results/discussion were given, the details were not taken in. Although the aims of the study were given in the introduction, the whole experiment was as though it was a surprise - you didn't know what was coming next, which I felt made it hard to concentrate and remember details. The inclusion of methods, results and discussion into just one section also made the section ridiculously long, thus making the reader lose interest. I feel that the authors should have structured the paper better. I believe that it was not necessary to include the discussion in with the results. Shorter, separate methods, results and discussion sections would have been appropriate. Despite the above, generally, when the methods when given they were detailed, and the statistical analyses used were clearly stated. Nowhere, however, does it state the conditions used for the pH, temperature, nutrient, and dissolved organic carbon stress for the first experiment. And when describing the method for the second experiment, it only states how they varied the pH and the temperature. This makes it difficult to repeat the experiment and to assess the reliability of the results. I thought the inclusion of a second experiment a year later was rigorous and helped the authors achieve more reliable results.

Authors' arguments and conclusions: The conclusion that herpes-like viruses are commonly associated with corals is well backed up by metagenomic analysis. Similarly, the fact that the viruses can be 'produced' by environmental stress such as pH, temperature and nutrients, is supposedly well supported by experiments. I say 'supposedly' because the results show this, however the authors do not state the conditions used to test this. They do not say by how much they raised the temperature, or altered the pH for example. Because of this, it cannot be seen if they

changed the parameters by a small or large amount, thus it is difficult to assess the results.

In the conclusion it is also stated that 'herpes-like viruses are commonly associated with corals and perhaps all Cnidarians'. I feel that the latter part of this statement is unfounded, as apart from the one coral species, they tested only one anemone genus, *Nematostella*, and one such *Hydra*. Although it was simply a suggestion that herpes-like viruses are associated with all Cnidarians, I feel it would have been more appropriate to suggest that further research be undertaken to investigate whether herpes-like viruses are associated with all Cnidarians.

Significance: This paper studies an important, newly emerging, area in coral microbiology. Marhaver *et al.* (2008) state that little is known about viruses that infect corals and their symbionts. Therefore any research which sheds light on this topic is significant and may add to the understanding of the mechanisms of coral disease and bleaching.

The results of this study show herpes-like viruses are present in corals and that temperature, pH, and nutrient stress induce the viruses to become more abundant. The authors state that the experimental design may have mimicked fragmentation and small-scale predation that commonly occurs on reefs, and therefore propose that these natural activities are stressful enough to produce production of herpes-like viruses on the reef and may result in short or long-term reductions in coral health. This study was also the first to use 'massively parallel' pyrosequencing for empirical experiments. The method worked well and the authors therefore suggest that this method can be used as a first step in identifying novel viruses in almost all systems.

Connections: Studies investigating the roles of viruses in corals have only begun to take off in the past few years. A 2007 paper by Davy and Patten investigated the diversity of viruses in *Porites* spp. and found a diverse assemblage of virus-like particles in the coral holobiont. An earlier study by Davy *et al.* (2006) attempted to determine whether viruses are present in three species of coral, different to the one used here, and in zoanthids and their zooxanthellae when thermally stressed. Similar to the present paper, they found evidence to support that thermally stressed corals are infected by viruses. Marhaver *et al.* (2008) also found that herpes-like viruses comprise a large percentage of the total sequences in the metagenome of *Diploria strigosa*. This current paper has brought many of these ideas together by not only exploring the diversity of viruses associated with the studied coral, but by also testing a suite of environmental variables to investigate in more depth the role these viruses might play in coral disease. Further studies should be conducted to investigate the effect of these virus-like particles on coral health, and to further look into how natural disturbances (including by humans, e.g. snorkellers) can induce these virus-like particles.

Overall judgement: This paper investigates a recently emerged idea that viruses are involved in coral disease. It sets about the investigation in a rigorous manner and puts the results into a wider context. However, the paper is let down by the structure.

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Davy, J. E., Patten, N. L. (2007). Morphological diversity of virus-like particles within the surface microlayer of scleractinian corals. *Aquatic Microbial Ecology* **47**: 37-44.
Marhaver, K. L., Edwards, R. A., Rohwer, F. (2008). Viral communities associated with healthy and bleaching corals. *Environmental Microbiology* **10**: 2277-2286.

Viruses have a major function in deep-sea ecosystems

Elizabeth Robertson

A review of the article:

Danovaro, R., 'Anno, A., Corinaldesi, C., Magagnini, M., Noble, R., Tamburini, C. and Weinbauer, M. (2008). Major viral impact on the functioning of benthic deep-sea ecosystems. *Nature* **454**: 1084-1087.

The authors describe the importance of viruses in the dynamics of deep-sea microbial communities and identify the paradox of there being a high microbial biomass in the deep-sea sediments, yet the deep-sea as a whole is food limited. Although no definite hypotheses are presented, the aim of the study seems to be to assess the impact of viruses on deep-sea sediment bacterial populations. The study sites are situated in the Atlantic, Pacific Oceans and the Mediterranean and Baltic Seas, where depths from the shelf break to the abyssal plains were sampled, actively excluding 'hotspots' such as hydrothermal systems or deep-sea reefs. Viral abundance, production and turnover were estimated, as well as prokaryotic production, burst size, biomass and abundance. The authors conclude an important role of viruses in the lysis of prokaryotes and, hence, release of dissolved Carbon and nutrients in these 'food-limited' environments, the Carbon released being held in the microbial loop rather than being transferred to higher trophic levels. It is additionally suggested that the substances released in the lysate are readily available for uptake by remaining heterotrophic prokaryotes and that the lysis of certain microbial groups may reduce competition for nutrients. Final conclusions identify the need for viral activities to be included in biogeochemical and nutrient cycling.

The purpose of the study was to bring the importance of viruses in the deep-sea sediments to the attention of a broad scientific community. The fact that this article has been published in the journal 'Nature' indicates it is accredited as being of high interest and importance to scientific progression. Accordingly, the tone of writing is indicative of a presumed knowledge of recent advancements in molecular and microbiological techniques, with methodologies largely explained (slightly disjointedly) in the supplementary text. The risk of having a paper published in a prestigious journal such as *Nature*, however, is that much of the methodology and reasoning behind the study is lost due to the authors being supplied with limited space in which to put across all their conclusions and data. The remaining text is largely 'groundbreaking' conclusions, ill-explained methods and a select few figures.

The objectivity of the study is clearly based on the impact of the viral component on prokaryotic assemblages in deep-sea sediments, however there is little reference to previous findings in the area, even those studies conducted by several of the present authors. The very fact that the article is published in *Nature* gives both study and authors a high credibility; this paper being seen to unarguably one of the broadest scientific audiences of any journal. The project is largely funded by the EU as part of the HERMES (Hotspot Ecosystem Research on the Margins of European Seas) project, which aims to increase our understanding of relationships between biodiversity and ecosystem functioning. Judging by the sheer amount of data, nature of locations and the equipment used (i.e. deep sea corers) the study had a considerable budget. The resources put into the investigation are obviously substantial and, judging by the global implications and importance of the conclusions being made seem well justified.

The methodology in the main article, as mentioned before, is obviously aimed at those of a scientific inclination. The methods are appropriate but occasionally poorly described (i.e. refer the reader to other papers for further information) for example the dilution technique and the exact purpose or description of mitomycin C in assessing the lysogenic fraction of prokaryotes. The former experiment may have induced some discrepancy by diluted virus and prokaryotic samples being incubated in small syringes. As described by Zobell and Anderson (1936), the confinement of marine microbes in small containers generally causes an enhancement of prokaryotic growth rate. Although the short space of time these samples were kept in the syringes may have had a negligible effect, it is advisable that this increased growth be taken into account. An additional issue with the dilution technique is that the paper the reader is referred to for a full method is that involving water sampling as opposed to sediment. In the present paper, no mention is made as to the additional steps needed to remove microbial components from particles of sediment.

Results are general and poorly explained, with the limited figures provided being a jumble of colour coding with little explanation of between-site variability in the text. Although some percentages are provided in the text to back up results, it is not apparent from those graphs included in the article as to the findings of the study. Figures in the supplementary information seem to provide more of an insight as to the actual results, with the majority of the findings being presented as broad conclusions and attention-grabbing statements. Statistical methods used are mentioned briefly as the final paragraph in the article but there is no evidence of the analysis being used in the main text. The results of the multivariate multiple regressions of environmental variables of viral abundance and production are provided in the supplementary text but require further explanation. Standard deviations of data are, however, indicated on most supplementary figures.

From the limited results explained in the text, it seems that the conclusions made are valid and well supported. Interestingly, the paper makes no attempt to refer to previous work of the present authors in this area, even to enable a comparison to the findings of this study. The main author has conducted extensive work on deep-sea sediment viral component yet only refers to one very similar study conducted in 2004 (Mei and Danovaro, 2004), which seems to have had less funding and less impact on the scientific community (due to the journal in which it is published). Commendably, it seems that the authors felt the importance of their conclusions needed exhibiting to the larger audience and so may have conducted this study in order to achieve a publication in *Nature*. The seeming large-scale implications suggested by the paper will undoubtedly be of interest to all walks of science (i.e. implications for importance of viruses in global biogeochemical cycling).

No further work is suggested; however, undoubtedly more work by the present authors in this area will be conducted. From observing the map provided in the supplement, it seems that those sites where experimentation occurred seem fairly coastal (despite some being over 5000m) or enclosed. It may have been interesting to take results from more open ocean sites (e.g. central Pacific, Sargasso Sea) to see whether the influence of coastal inputs can, or has affected these deep sediments.

This study seems to be one of the largest advances, and indeed largest investigation conducted in the study of deep-sea virology and the conclusions drawn from the data do seem to have important implications. Data sampling has been extensive and no doubt this paper will be referenced by many more in the future. Wider implications may include the impacts of viruses in other sediments. The methods used here to estimate production and abundance and the relationships between prokaryotes and viruses may also be applied to other ecosystems to assess the importance of the viral shunt to the community and Carbon flow.

Similar studies have been conducted but usually on a much smaller scale but have generally drawn similar conclusions to each other; however, this study's conclusions seem somewhat contradictory to those previous studies by these authors. This study emphasises the importance of viruses in sediments and at the sediment-water interface, yet other studies (Danovaro and Serresi, 2000) state that these viral-prokaryote interactions are much less significant, and that those interactions in the pelagic zone are more important in these cycles. Although this may be true, it seems odd that no reference is made to the previous study in this article, if only to disprove the findings of it. A similar situation is true of a second paper (Danovaro et al, 2002), which focuses on Mediterranean deep sediments. Despite the present study indicating a high virus to prokaryote ratio, the 2002 paper indicates that in some Mediterranean sediments, bacteria are equal to, or even outnumber viruses. It seems strange that this study was not mentioned in this 2008 paper, especially as there is a large amount of study sites located in the same sea. It may even seem like there is a bias toward what the authors wanted to find and report.

Overall, the paper is one of importance and it seems creditable that the authors may have wanted to push a previous smaller study into the wider scientific world but publishing in *Nature*. Although the methodology can seem confusing and the results slightly disjointed and almost irrelevant at times, the conclusions drawn are important for the global cycling and recycling of compounds. Despite some contradictions of previous work, the importance of the study is obvious; the impact of tiny viruses on such a massive ecosystem is certainly one not to be ignored in the future of scientific research.

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